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VIRUSES IN WASTE, RENOVATED, AND OTHER WATERS

Gerald Berg, et al

Environmental Protection Agency
Cincinnati, Ohio

1970

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LITERATURE ABSTRACTS

ENVIRONMENTAL PROTECTION AGENCY
Water Quality Office
Cincinnati, Ohio

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VIRUSES IN WASTE, RENOVATED, AND OTHER WATERS

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Virology Section
Advanced Waste Treatment Research Laboratory
Water Quality Office
Environmental Protection Agency
Cincinnati, Ohio

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LITERATURE ABSTRACTS

VIRUSES IN WASTE, RENOVATED, AND OTHER WATERS

(Anonymous) (1970). *Microbiology. Waterborne Outbreaks.* **J WATER POLLUT CONTRL FED, 42(6):** 1057-77.

As part of a general literature survey of the microbiology of water, some methods for detecting viruses in water are reviewed, and some data on virus recoveries from waste and recreational waters are described.

Albano, A., DeDonato, S. (1969). *Contamination of Surface Water: Virological Aspects.* **IG MOD, 62**:846-70. Italian.

Waterborne viral infections are discussed, and the literature on the presence and survival of viruses in sewage and surface water and their behavior during treatment procedures is reviewed.

The absence of waterborne epidemics and sporadic cases are discussed. A need for chlorine doses well above those commonly used is discussed. The need for a good indicator of viral contamination is noted.

Albertsson, P.-A. (1970). *Partition of Cell Particles and Macromolecules in Polymer Two-phase Systems.* **ADV PROTEIN CHEM, 24**:309-41.

The polymer two-phase system developed for the separation of cell particles and macromolecules is extensively reviewed. The adaptation of the system to the concentration and purification of viruses from dilute and concentrated systems is discussed.

Bagdasaryan, G. A. (1970). *Comparative Survival of Enteroviruses in Polluted Waters.* **ZH MIKROBIOL EPIDEM IMMUN, 8(8)**:96-103. **Translation presently not available.** Russian.

Brisou, J. (1968). *Viral and Parasitic Pollution of Littoral Waters and Its Consequences on Public Health.* **BULL WHO, 38**:79-118. French.

The consequences of pollution of coastal waters with sewage are evaluated, and the importance of viruses in such pollution is noted as is the importance of bacteria, fungi, and parasites that are also present in sewage.

The absence of international or uniform standards among coastal nations for sewage discharged into the seas is discussed.

Bondarenko, V. I., Zykova, O. L., Grigoryeva, L. V. (1968). *Dissemination of Enteroviruses Among the Population.* **VRACH DELO, 10(10)**:89-91. Russian.

One hundred and twelve strains of enteroviruses were recovered from 160 samples of sewage in Kiev.

Thirty-four echovirus strains and 61 coxsackievirus strains were recovered from the feces and oral and nasal washings from 120 healthy children and from 170 children ill with intestinal disorders.

Butrico, F. A., Dunsmore, H. J., Lischer, V. C., Ludwig, H. F., Nevins, F., Reid, G. W., Romer, H., Sproul, O. J., Berger, B. B., Chairman. Committee on Environmental Quality Management (1970). *Engineering Evaluation of Virus Hazard in Water.* **J SAN ENGIN DIV, ASCE, 96**:SA1:111-61.

This is a report by the Committee on Environmental Quality Management of the Sanitary Engineering Division of the American Society of Civil Engineers.

The Committee believes the extent of the hazard presented by viruses in water is still uncertain, but that the virus threat will increase. The Committee notes that good water sanitation practices require a good water source that will not overload treatment facilities, because even a temporary breakdown of treatment processes may permit viruses to reach finished waters.

The Committee believes that Public Health Service maximum permissible fecal coliform concentrations serve as an indicator of treatment process performance, but may not be valid during pulses of heavy pollution.

The Committee recommends that water treatment facilities reduce turbidity to below 0.1 Jackson unit, and provide an HOCl residual of 1 mg/liter after 30 minutes of contact. For waters with a pH above eight, the Committee recommends a 50% increase of the residual chlorine level or of the contact time.

Carazzone, M. M., Vanini, G. C. (1969). *Experimental Studies on the Effect of Ozone on Viruses. I. Effect on Bacteriophage T1.* **G BATT VIROL IMMUN, 62(11-12)**:828-39. Italian.

Ozone concentrations of 0.5 and 0.55 mg/liter completely inactivated coliphage T2 after five minutes of contact.

Carlson, S. (1969). *Virus Occurrence in Raw Sewage Sludge and Effluent of a Biological Clarification Plant.* **ZBL BAKT ORIG, 212(12)**:50-60. German.

In the course of a year, enteroviruses were demonstrated regularly in five-ml volumes of raw sewage in Berlin and in a Berlin suburb. The smallest volumes of sewage containing viruses were 0.1 ml in summer months and 0.5 ml in winter months. Viruses were rarely recovered in even 50-ml volumes of raw sewage from a convalescent home for elderly persons.

Preferential adsorption of viruses to sewage sludge did not appear to occur.

Tests with attenuated poliovirus 1 performed in a clarification plant consisting of an oxidation ditch, secondary sedimentation tanks, and tower-type percolating filters resulted in a virus reduction of about one log.

Chang, L. W., O'Brien, T. F. (1970). *Australia Antigen Serology in the Holy Cross Football Team Hepatitis Outbreak.* **LANCET, 2(7)**:59-61.

Australia (Au) antigen was not detected in sera from patients by either agar-gel diffusion or by complement fixation tests in a large-scale, well-defined, simultaneous point-source outbreak of short-incubation viral hepatitis. No Au antibody was detected by agar-gel diffusion. The serums from most icteric patients had high titers of anticomplementary activity which declined in convalescence. Serum anticomplementary activity in infectious hepatitis seemed related to the degree of illness but not to Au antigen.

Chaudhuri, M., Englebrecht, R. S. (1970). *Removal of Viruses from Water by Chemical Coagulation and Flocculation.* **J AMER WATER WORKS ASSN, 62(9)**:563-7.

Coagulation and flocculation with $Al_2(SO_4)_3$ removed 98 to 99.9% of coliphages T4 and MS2 from seeded wastewater and wastewater effluent. Calcium and magnesium, in concentrations up to 50 mg/liter, did not reduce the efficiency of removal, but organic matter did.

Cationic polyelectrolytes Primafloc C-7 and Catafloc removed 99.9% of the viruses when used as prime coagulants.

Clarke, N. A., Berg, G., Liu, O. C., Metcalf, Th., Sullivan, R., Vlassoff, L. T. (1970). *Occurrence of Viruses in Water.* **ARCH HYG BAKT, 154(12):**314-18. German.

This is a report of the AWWA Committee on Viruses originally published in the Journal of the American Water Works Association [61(10):491-4]. It presents the current status of the problem of viruses in water, it summarizes what is known about the removal of viruses by treatment processes, and it discusses the virus indicator problem, and some current research needs.

The committee noted that water can be freed from viruses by clarification (coagulation, sedimentation, and filtration) followed by disinfection, and that disinfection can best be achieved in waters with turbidities of less than one Jackson unit. With such low turbidities, the committee noted, disinfection probably requires not more than 1 mg/liter of HOCl after a 30-minute holding period.

Cookson, J. T., Jr. (1970). *Removal of Submicron Particles in Packed Beds.* **ENVIRON SCI & TECHN, 4(2):**128-34.

The influence of physical parameters on the removal of viruses (colloidal particles) was evaluated, and a model presented correlating physical filtration parameters to removal efficiency. Removal of coliphage T4 was studied in packed beds of activated carbon. The chemical characteristics of the system were maintained at conditions optimum for virus attachment, and the physical characteristics of the system were varied. Virus removal was interpreted by a physical model based on particle transport by diffusion in a flowing fluid. Virus removal closely followed the transfer model which may be usable for predicting the effects of flow rate, bed height, void volume, and bed grain size on removal efficiency.

Criscuolo, A., DeDonato, S., Albano, A. (1968). *Recovery of Polioviruses and Other Cytopathic Enteric Viruses in Sewage of Certain Areas in Lombardy.* **ANN SCLAVO, 9(6):**886-99. Italian.

Of 54 sewage samples examined from provinces in Lombardy, 37 contained viruses. Twenty-six of these were polioviruses. All were vaccine-like. A live poliovirus vaccine program had been initiated three years earlier. Simultaneous stool studies indicated a low incidence of wild-type polioviruses in the provinces.

Dhillon, T. S., Chan, Y. S., Sun, S. M., Chau, W. S. (1970). *Distribution of Coliphages in Hong Kong Sewage.* **APPL MICROBIOL, 20(8):**187-91.

Coliphage counts in Hong Kong sewage from 11 different areas ranged from 36 - 15,900 PFU/ml. Urban sewage generally contained more coliphages and a greater variety of morphological plaque types than rural sewage.

About half of 77 of the isolates tested multiplied in both **Escherichia coli** K-12 and in **E. coli** B. About 32% of the 77 were male specific, and the remaining 18% were K-12 specific but sex-indifferent.

Diesch, S. L. (1970). *Disease Transmission by Waterborne Organisms of Animal Origin.* **BOL SANIT PANAM, 69(10):**314-29. Spanish.

Infectious diseases of animal origin in man and animals have been associated with water transmission. Water transmission of animal viruses is almost wholly unstudied, and with available methods, the significance of disease transmission via water cannot be measured.

The risks of contracting zoonotic diseases through water exposure may be increasingly significant in the future. Water is only one method of disease transmission, but water is essential for life and all men and animals are exposed to water. Animal waste disposal and the effects on the environment increase with livestock concentration. Future population growth and new developments will change occupational and recreational methods, and these factors will upset the ecological systems in nature that exist today. What will happen in the future as a result of these ecological changes cannot be predicted.

The future effect of changing agricultural practices, growth and concentration of animal and human populations, and man's increasing exposure to water will challenge scientific disciplines to assess interrelated disease associations.

DiGirolamo, R., Liston, J., Matches, J. R. (1970). *Survival of Viruses in Chilled Frozen, and Processed Oysters.* **APPL MICROBIOL, 20(7):**58-63.

Whole and shucked Pacific and Olympia oysters, seeded with 10^4 PFU of poliovirus 1/ml, were refrigerated at five and -17.5 C. Samples were assayed for virus content weekly for up to 12 weeks. The poliovirus survived in refrigerated oysters for 30 to 90 days, depending upon temperature.

Samples of whole and shucked Pacific oysters seeded with 10^4 PFU of the poliovirus/ml were heat processed in four ways: stewing, frying, baking, and steaming. Survival varied from seven to 10%. Heat penetration studies showed

that the internal temperatures in the oysters were not sufficient to inactivate all of the virus. These results suggested that not only fresh but also refrigerated and cooked oysters can serve as vectors for the dissemination of virus disease if shellfish are harvested from a polluted area.

Duff, M. F. (1970). *Isolation of Ether-Resistant Enteroviruses from Sewage: Methodology.* **APPL MICROBIOL, 19(1):**120-7.

Viruses exist in sewage as free or adsorbed virions. Poliovirus 1 and coxsackievirus A8 did not adsorb onto cotton fibers of sewer swabs.

Sewer swabs filter out bound viruses over several days and represent grab samples for that period of time.

A simple method for preparation of sewage inocula for virus isolations is described which recovers the bound ether-resistant virus fraction with fluorocarbon. An ultracentrifuge was not required. Between 60 and 80% of sewer swabs yielded viruses by this method. Between 84 and 96% of sewer swab ultracentrifuged fluorocarbon-treated eluates yielded viruses. Virus concentrations sometimes reached a level of one infectious particle per 0.5 ml of sewage.

Faulkner, R. S., Van Rooyen, C. E. (1969). *Studies on Surveillance and Survival of Viruses in Sewage in Nova Scotia.* **CAN J PUB HEALTH, 60(9):**345-50.

Viruses were recovered from 77 of 143 grab samples of sewage concentrated by ultracentrifugation. No recoveries resulted from inoculation of suckling mice.

Echovirus 6 was more readily recovered in human amnion cultures than in monkey kidney cultures. Coxsackieviruses B4 and B5 were more readily recovered in monkey kidney cultures than in human amnion cultures.

Correlation could not be demonstrated between virus recovery and the socioeconomic level of the population.

No enterovirus epidemics were reported in the community. Three polioviruses, one vaccine-like, were recovered from sewage although no clinical poliomyelitis occurred and live attenuated vaccine was not being administered.

Gavan, D. T., Nutt, J. W. (1970). *An epidemic of Waterborne Infectious Hepatitis in France.* **ARCH ENVIRON HEALTH (Chicago), 20(4):**523-32.

In an outbreak of infectious hepatitis in a French municipality, epidemiologic evidence pointed to an extensively contaminated municipal distribution system as the vehicle. The median incubation period in Americans living in the community was 38 days with a range of 29 to 57 days. Prolonged exposure apparently did not occur. There was no evidence that the length of the incubation period was influenced by the chlorination practiced. In-line chlorination, however, may have been responsible for the low incidence of diagnosed hepatitis in an Army housing area receiving water from the municipal distribution system.

Gerth, J.-J., Bassler, S., Steinitz, H. (1970). *Significance of Instructional Swimming Facilities in the Epidemic Occurrence of Plantar Warts.* **MED WELT, 8(2):**298-303. German.

An outbreak of plantar's wart infections in 1965 to 1966, believed to have been swimming pool-associated, is described. Two-hundred-and-forty school children were affected along with 35 family contacts. The total case rate was about 14.5% with about equal distribution between males and females. The rate among 10 to 11 year olds was 28.7%.

Goncharuk, E. I., Grigoryeva, L. V., Bei, T. V., Shulyak, E. V., Korchak, G. M. (1970). *Removal of Intestinal Viruses and Bacteria from Sewage by a Circulating Oxidizing Canal.* **GIG SANIT, 35(1):**32-6. Russian. **(Translation-HYG & SANIT, 35(1):**36-41, 1970.)

Treatment of sewage in a circulating canal for two days removed coxsackievirus B5 and echovirus 19 in 24 and 48 hours respectively. A coliphage was removed in 12 hours when it was initially present in a concentration of 50 PFU/ml, and in 16 hours when it was initially present in a concentration of 6,000 – 7,000 PFU/ml.

Pathogenic **Escherichia coli** were recovered from sewage and sludge for three to seven days when its initial concentration was one million/liter, and for 15 to 18 days when its initial concentration was 100 million/liter. Coxsackievirus B5 was recovered from activated sludge for three days and echovirus 19 for five days. Coliphage were detectable for 15 to 25 days depending upon their initial concentration. The authors concluded that sewage effluents must be disinfected.

Grady, G. F. (1968). *The Prevention of (Viral) Hepatitis.* **DIS-A-MONTH (7)**:1-31.

The nature of hepatitis, its diagnosis and treatment are discussed together with its etiology and transmission. This paper is primarily a review for clinicians.

Grindrod, J., Cliver, D. O. (1969). *Limitations of the Polymer Two-Phase System for Detection of Viruses.* **ARCH GES VIRUSFORSCH, 28**:337-47.

The aqueous polymer two-phase technic was a significant aid to detection of each of the poliovirus types, coxsackievirus B3, and, in the presence of maintenance medium only, coxsackievirus A9. The technic was worse than no treatment of samples for recovering coxsackievirus B2 and echovirus 6. The lower phase containing dextran sulfate, into which the viruses concentrate, was strongly inhibitory to these two viruses and to influenza A (PR8) virus also.

Grinstein, S., Melnick, J. L., Wallis, C. (1970). *Virus Isolations from Sewage and from a Stream Receiving Effluents of Sewage Treatment Plants.* **BULL WHO, 42**:291-6.

A method for concentrating viruses by adsorption onto insoluble cross-linked maleic anhydride polyelectrolytes was used to survey sewage and a stream receiving sewage effluents in a residential area of Houston, Texas. The virus flow at different points along the stream varied from 304,000 to 6,014,000 PFU/ minute on a single day. From 84 samples, each of one US gallon, 14,520 viruses were recovered. Most of the viruses were echovirus 7 and polioviruses 1, 2, and 3, some with characteristics of virulent wild strains.

Hahn, R. G., Hatlen, J. B., Kenny, G. E. (1970). *Comparative Poliovirus Permeability of Silver, Polycarbonate, and Cellulose Membrane Filters.* **APPL MICROBIOL, 19(2)**:317-20.

The permeability of silver, polycarbonate, and cellulose filters to poliovirus 1 suspended in water, in salt-containing, and proteinacious solvents was determined.

The suspending medium was a critical factor in permeability of cellulose filters. The virus did not pass through these filters in either water or salt solution. Proteins increased filterability.

The virus permeability of silver and polycarbonate filters was independent of the suspending medium. Apparently, pore size alone determined their

permeability towards poliovirus. Electrostatic forces between filters and the virus did not appear to play a significant role. The silver and polycarbonate filters may be useful tools for separating viruses from bacteria and fungi.

Hartman, D. (1968). *Epidemic Occurrence of Echovirus 6 and Echovirus 30 Illnesses During the Late Summer 1967 in Berlin.* **ZBL BAKT ORIG, 207(5):**141-4. German.

Aseptic meningitis associated with echovirus 6 and echovirus 30 occurred in the late summer and fall of 1967 in West Berlin. Echovirus 6 was found in West Berlin sewage during all of 1967, but echovirus 30 was found only sporadically until the summer, after which it was recovered from sewage regularly. Other echoviruses, polioviruses, and coxsackieviruses were also recovered from the sewage sporadically or regularly, but were not associated with aseptic meningitis.

Hill, W. F., Jr., Hamlet, F. E., Benton, W. H., Akin, E. W. (1970). *Ultraviolet Devitalization of Eight Selected Enteric Viruses in Estuarine Water.* **APPL MICROBIOL, 19(5):**805-12.

The effect of ultraviolet (UV) radiation on the devitalization of eight selected enteric viruses suspended in estuarine water was determined. Polioviruses 1, 2, and 3, echoviruses 1 and 11, and coxsackievirus A9 exhibited similar devitalization rates.

The devitalization rates of coxsackievirus B1 and reovirus 1 were different from those of the other viruses. This difference in devitalization rates of coxsackievirus B1 and reovirus 1 was attributed primarily to the frequency distribution of single and aggregate virions, the geometric configuration, the size of the aggregates, and the degree of aggregation. UV can be an effective virucide, and can provide a reliable safety factor in disinfecting estuarine water.

Khor'kov, I. A., Voinov, S. I., Klientova, G. S. (1969). *Survival Time of Foot-and-Mouth Disease Virus in the Sewage of Biological Products Factories.* **VETERINARIIA, 5(5):**91-4. Russian.

The survival time of foot-and-mouth disease virus in bits of infected tissue placed in the wastewater of the production area of a biological products factory was nine days at a water temperature of 30C. At a water temperature of 25C, the foot-and-mouth disease virus survived for eight to 13 days.

To destroy foot-and-mouth disease virus quickly in biological products factories, waters had to be heated for 30 minutes at 90C.

Korchak, G. I. (1970). *A Method for Detecting Enteroviruses in Activated Sludge.* **LAB DELO, 3:**170-2. Russian.

Efficient elution of coxsackievirus B6, and echoviruses 2 and 19 from seeded activated sludge were obtained with meat peptone, and 10% Na_2HPO_4at pH 8.6 to 8.8. Coxsackieviruses B4 and B5 were less efficiently eluted. Water, meat peptone alone, 10% sodium phosphate alone, and phosphate buffer alone, all used at a variety of pH levels, eluted viruses inefficiently.

Kott, Y., Ben Ari, H., Buras, N. *The Fate of Viruses in a Marine Environment.* **"Advances in Water Pollution Research," edited by S. H. Jenkins. Proceedings of the 4th International Conference held in Prague 1969. Pergamon Press, Oxford, England and New York, New York** (1969), 823-9.

Escherichia coli bacteriophages attacked their specific hosts, but not **Aerobacter** phages present in sea water.

Bacteriophages were destroyed by the natural killing properties of sea water, but in contrast to the rapid destruction of bacteria, the phages were destroyed slowly. Coliphages appeared to be as resistant to the environment as enteroviruses. Thus, coliphages may serve as valid models for evaluating the fate of viruses in marine environments.

Kuwert, E., Hoher, P. G., Primavesi, C. A. (1970). *Intratypic Differentiation of Poliovirus Strains Isolated from Sewage: Consequences for the Practice of Oral Vaccination.* **ZBL BAKT ORIG, 215:**16-25. German.

Thirty-five poliovirus strains isolated from sewage before, during, and after the outbreak of a small poliomyelitis (type 1) epidemic in Gelsenkirchen-Essen in 1968 were characterized by three different method of intratypic differentiation (rct/40, MS and seromarker), and the results were correlated to the course of the disease and its control by oral vaccination. Virulent poliovirus was isolated nine days before the first clinical case became evident.

Twenty-two children aged 1 to 14 years and one adult with no previous vaccination record exhibited neurological symptoms. The epidemic slowed significantly after trivalent oral vaccination was undertaken. The last neurological case occurred two to three weeks after the beginning of the vaccination campaign. Thus, trivalent oral vaccination appeared highly effective for the control of the disease.

The observations emphasize the value of isolation and intratypic characterization of poliovirus strains from sewage lines as potent tools in a poliomyelitis surveillance program. Regular monitoring of sewage during summer and fall may afford early recognition of an imminent poliomyelitis epidemic. Emergency oral vaccination is recommended after the occurrence of one clinical case and repeated isolations of virulent viruses from sewage.

Lund, E. *Observations on the Virus Binding Capacity of Sludge.* **Presented at the Fifth International Water Pollution Research Conference, July-August, 1970. Proceedings to be published by Pergamon Press Ltd., Spring 1971.**

Observations accumulated over a number of years on the recovery of viruses from sewage and treated effluents by the phase separation method, and by other methods, are described. Relative recoveries of viruses from digester sludge, sewage influents, and treated effluents are compared.

Data suggesting that viruses in sewage and sludge are bound to large particles are discussed.

Mats, L. I., Korsh, L. E. (1967). *Circulation of Pathogenic Microorganisms And Viruses in Soil, Water, and Air.* **HYG & SANIT, 32(9):**415-19.

The survival of viruses and other microorganisms in the environment is reviewed. The quantities of viruses in sewage and treated effluents are discussed. The extended survival of enteroviruses in sludge, soils, water, and on contaminated vegetables is noted. The capability of some treatment methods and some disinfection methods for destroying enteroviruses is discussed.

McCabe, L. J., Symons, J. M., Lee, R. D., Robeck, G. G. (1970). *Survey of Community Water Supply Systems.* **J AMER WATER WORKS ASSN, 62(11):**670-87.

The results of a nationwide Community Water Supply Survey by the U. S. Public Health Service in 1969 are presented. A total of 969 supplies serving more than 18 million people were surveyed.

Only 59% of the systems delivered water that met the USPHS 1962 Drinking Water Standards.

Smaller water supply systems less frequently met Drinking Water Standards than larger systems did.

Twelve percent of the supplies surveyed showed coliform densities greater than those permitted by the Drinking Water Standards.

Deficiencies in chemical quality, facilities, management, and surveillance are also discussed.

McLean, D. M., Brown, J. R. (1968). *Marine and Freshwater Virus Dispersal.* **CAN J PUB HEALTH, 59(3):**100-4.

Poliovirus 2 (vaccine strain) persisted at undiminished titer in freshwater for 12 days at 4C, but infectivity declined 100-fold in seawater over this period at 4C, and was undetected in seawater after five days at 25C.

Infectivity of this virus persisted at beaches and in open water for five to ten minutes both in seawater and freshwater. The virus was recovered after 65 feet of lateral drift at a freshwater beach, and after 25 feet of drift at a sea beach. Virus dispersal was relatively unaffected by wave actions.

With adequate mixing, inactivation of the virus occurred following addition of high residuals of chlorine to creeks to which the virus had been added.

Moore, M. L., Ludovici, P. P., Jeter, W. S. (1970). *Quantitative Methods for the Concentration of Viruses in Wastewater.* **J WATER POLLUT CONTR FED, 42(2):**Suppl-R21-8.

Wastewater was seeded with poliovirus 1 and the recovery efficiencies of aluminum hydroxide adsorption, and membrane filtration were compared.

Serum pretreatment of Millipore AP 20 pads eliminated loss of virus during wastewater clarification, but similar treatment of Millipore GA 0.22 μ membranes only reduced the loss from 76 to 31%. No loss of virus occurred when untreated Gelman GA-8, 0.20 μ membranes were used similarly to remove organisms from wastewater. Virus loss also occurred during the final concentration step when virus failed to absorb to the Millipore 0.45 μ membranes.

Two membrane adsorption procedures for concentrating virus—in which, prior to filtration through the membranes, protamine sulfate or an anion exchange resin removed from wastewater components that interfered with virus adsorption to the membranes—were compared with the aluminum hydroxide [$Al(OH)_3$] technic. The most efficient procedure for recovering viruses was the $Al(OH)_3$ method which yielded 81 to 100% recovery as compared with only 23 to 38% for membrane adsorption when either protamine sulfate or resin treatment of the wastewater preceded filtration. The $Al(OH)_3$ procedure not

only more efficiently detected seeded poliovirus 1 at low input levels but more effectively detected low levels of indigenous viruses in wastewater.

Mose, J. R., Dostal, V., Wege, H. (1970). *Inactivation of Viruses by Wastewater Protozoa.* **ARCH HYG BAKT, 154(12):**319-29. German.

The influence of **Tetrahymena pyriformis** and **Acanthamoeba hartmanella** strain A1 on myxoviruses, poxviruses, and enteroviruses was examined. **Tetrahymena** inactivated influenza and vaccinia viruses, but not poliovirus 1.

The hemagglutinin of influenza viruses A and B were destroyed only by living protozoa. Dead **Tetrahymena** inactivated influenza virus and vaccinia virus. Proteolytic enzymes of these protozoa may be the cause of inactivation.

By means of fluorescent antibodies, it was demonstrated that influenza viruses adsorb to the surfaces of protozoa and are later also found in the nutrient vacuoles. By immunofluorescence, vaccinia virus was also detected in the vacuoles of **Tetrahymena.**

Acanthamoeba hartmanella did not destroy poliovirus 1, and caused little decrease in infectivity of influenza and vaccinia viruses after more than a week. It did not affect the hemagglutinin of influenza virus either.

Muzzi, A., Borgioli, A. (1969). *Water Pollution with Viruses.* **ANN SANIT PUBBLICA, 30(3-4):**171-205. **Translation presently not available.** Italian.

Muzzi, A., Borgioli, A. (1969). *Water Pollution of a Viral Nature.* **ANN SANIT PUBBLICA, 30(5-6):**341-77. **Translation presently not available.** Italian.

Nupen, E. M. (1970). *Virus Studies on the Windhoek Wastewater Reclamation Plant (South-West Africa).* **WATER RES, 4(10):**661-72.

The $TCID_{50}$ method was superior to the PFU method for detecting and quantitating viruses in sewage.

Comparable concentration and recovery of viruses occurred in parallel statistical tests of the two-phase polymer separation method and the alginate ultra-filtration method for quantitative concentration of viruses.

These methods of concentration were used for testing virus removal in the advanced wastewater treatment plant at Windhoek, South Africa. Although

enteroviruses and reoviruses entered the sewage purification plant at levels as high as 20,000 $TCID_{50}$/liter, no viruses were recovered from the final product water.

O'Connor, J. T., Kapoor, S. K. (1970). *Small Quantity Field Disinfection.* **J AMER WATER WORKS ASSN, 62(2)**:80-4.

The effectiveness of halazone tablets and globaline tablets for the disinfection of small water supplies was re-evaluated. Globaline (which releases I_2) replaced halazone (which releases OCl^-) as a small water supply disinfectant during the second World War. Globaline quickly inactivated **Endamoeba histolytica** cysts and halazone did not.

In recent studies, globaline did not dissolve quickly after long storage and produced some taste and odor problems.

Changing the alkaline buffer of halazone to an acidic buffer which would convert OCl^- to HOCl and thereby increase the cysticidal effectiveness of halazone was suggested. Increasing the pH of globaline to eight, which would hydrolyze the I_2 to the even more virucidal HOI, was also suggested.

Osherovich, A. M., Chasovnikova, G. S. (1969). *Results of a Virologic Investigation of Environmental Sources.* **HYG & SANIT, 34(3)**:424-7.

Sewage, effluents, raw waters, swimming pools, finished waters, and environmental objects were tested for viruses.

Sewage was sampled by the gauze pad method and secondary effluents were passed through filter paper and then through an ion-exchange column of Soviet-made resin AV-17 from which viruses were eluted. Raw waters were also tested by the resin method, as were samples of sand, soil, and sludges.

Fifty-three of 78 sewage samples contained enteroviruses, about half polioviruses. A large immunization program with oral poliovirus vaccine had recently been concluded.

Viruses were recovered from primary and secondary effluents, but not from sludges.

No viruses were detected by a one-time investigation of water samples taken at a bathing pond and a swimming pool. But single water samples from two children's sections of swimming pools in August and September of the same year yielded four strains of coxsackievirus B1, although the chemical and bacteriological indices of the water were satisfactory.

No viruses were detected in 22 sand samples from children's sand pits in September and October.

No viruses were recovered from washings from toys, tables, tableware, kitchenware or the hands of personnel in two kindergartens although viruses were found in the feces of 25% of the children. Samples had been taken after thorough cleaning of the premises and equipment. In a third children's institution, washings from a toy, collected one month after termination of an outbreak of disease caused by coxsackievirus B6, yielded the same virus. The virus was not detected in the children's feces at this time.

Raska, K., Helcl, J., Jezek, J., Kubelka, Z., Litov, M., Novak, K., Radkovsky, J., Sery, V., Zejdl, J., Zikmund, V. (1966). *A Milkborne Infectious Hepatitis Epidemic.* **J HYG EPIDEM MICROBIOL AND IMMUN, 10:**413-28.

An explosive epidemic of infectious hepatitis in and around Jablonec and Nisou in Czechoslovakia began in April 1963 and ended in June 1963. Hepatitis virus was believed spread by milk and dairy products contaminated by polluted water from a dairy's water supply.

Morbidity in the area served by the dairy (0.7%) was significantly higher than in the area not supplied by this dairy (0.09%). In the exposed area, 424 persons became ill; 40.3% of these were males and 59.7% females. The highest incidence was in the 15 to 20-year old group. There were very few contact infections, apparently because of mass administration of gamma globulin, especially to children.

Rennie, T. F. (1970). *Infectious Hepatitis: Report of an Outbreak, Apparently Waterborne, in an Institution.* **MED J AUSTRALIA, 2(7):**135-6.

An outbreak of infectious hepatitis occurred in an institution for the intellectually handicapped. There were 13 cases, 11 of which occurred within nine days.

Circumstantial evidence suggested this was a waterborne outbreak. There was no evidence that any person suffering from, or incubating, infectious hepatitis lived in, worked in, or visited the institution within the incubation period of the disease.

The outbreak was explosive indicating a common vehicle. Food handling, cooking, and storage facilities revealed no contamination of foodstuffs. The water supply, however, was frequently grossly contaminated by fecal organisms, probably of human origin.

Untreated polluted creek water was widely reticulated throughout the building and grounds and was probably consumed frequently.

Schafer, E. (1970). *Virus Detection in the Field of Water Hygiene.* **ARCH HYG BAKT, 154(12):**299-313. German.

Control of virus pollution in waste and other water is gaining increasing interest. During the summer, high enterovirus concentrations occur in water, but methods are available for totally removing them. Development of methods for detecting small amounts of viruses in large volumes of water is of paramount importance.

Schierbaum, L. (1968). *Combined Membrane and Alginate Filtration for Virus Concentration in Water.* **ZBL BAKT ORIG, 207(4):**453-6. German.

A method is described which decreases clogging of alginate filters by sewage effluents. The method consists of prefiltration of effluent through a paper filter to remove coarse debris, centrifugation at 12,000 rpm for 30 minutes, and filtration of the clarified effluent through a membrane filter and an alginate filter arranged in tandem.

Volumes of more than 500 ml were accommodated by this system.

Seigneurin, R., Causse, G., Magnin, R., Renaudet, J. (1968). *Viruses in River Water and in Non-Purified Effluents that Flow into Them.* **REVUE HYG MED SOC, 16(5):**415-32. German.

From July 1965 to April 1967, 44 viruses were recovered from 146 river and sewage effluent samples taken along the L'Isere and Grenoble Rivers, and from 104 samples taken directly from two sewers. More recoveries were made from gauze pads than from grab samples.

Thirty-six of the viruses recovered were adenoviruses, mostly types 2 and 7. The remaining viruses were reoviruses and enteroviruses.

The polioviruses and adenoviruses excreted by hospital patients were of the same types as those recovered from the hospital sewer and from the river. Adenoviruses were recovered throughout the year, but especially in summer and autumn. Polioviruses and coxsackieviruses were recovered mostly in autumn and winter.

Shuval, H. I., Fattal, B., Cymbalista, S., Goldblum, N. (1969). *The Phase-Separation Method for the Concentration and Detection of Viruses in Water.* **WATER RES, 3**:225-40.

The phase separation method for detecting and concentrating enteroviruses in water was refined. Several liters of water containing viruses were mixed with sodium dextran sulfate, polyethylene glycol and NaCl in which, after detention in a separatory funnel, two phases occurred. The small bottom phase concentrated the viruses and was drained off. The procedure was repeated to achieve a concentration factor of 500. One to two virus infectious units/liter of sample were detected.

With this recovery system, viruses were detected in most raw sewages and in effluents from treatment plants. A maximum of 11,184 PFU/liter were detected in raw sewage. Viruses were detected in two samples of water from streams, and in two samples of drinking water from a shallow municipal well.

All three types of polioviruses, including both vaccine and wild strains, were detected in sewage samples. Echoviruses and coxsackieviruses were also detected. As many as four different virus types were detected in a single sewage sample.

Primary rhesus kidney cells were about four times more sensitive than BSC 1 cells for detecting enteroviruses in sewage.

Shuval, H. I. *Detection and Control of Enteroviruses in the Water Environment.* **"Developments in Water Quality Research," edited by H. I. Shuval. Proceedings of the Jerusalem International Conference on Water Quality and Pollution Research, June 1969. Ann Arbor-Humphrey Science Publishers, Ann Arbor, Michigan, U.S.A. (London, England)** (1970), 47-71.

The phase separation method (dextran-polyethylene glycol) concentrated enteroviruses in water and wastewater by a factor of 500, and detected one or two PFU/liter of water.

In four cities in Israel, enteroviruses were detected in the sewage in all seasons of the year. The average concentration was 1,050 PFU/liter, but concentrations varied widely. Up to four different enteroviruses were detected in a single sewage sample. Enteroviruses were also detected in two of 70 drinking water samples tested. These two samples came from a polluted municipal well suspected as the source of an infectious hepatitis outbreak.

Enteroviruses were found in surface waters in five of 37 samples tested, once 25 km downstream from the main sewage outfall.

Primary settling did not remove viruses from sewage, and little or no removal occurred in a biological filtration plant although the plant was operating

normally and reduced coliform counts by 92%. An oxidation pond with a 20-day retention time removed about 67% of the virus and about 80 to 90% of the coliforms.

Eleven mg/liter of chlorine applied to sewage effluent reduced the enterovirus concentration by one log in the time it took to reduce the coliform concentration by five logs.

Enteroviruses were detected in 14 of 48 samples of seawater, in two instances 1.5 km from the nearest sewage outfall. Radioactive tracers indicated 1:1,000 dilution of the sewage at that point.

A marine antiviral agent (MAVA) was detected in water from the Mediteranean and Red Seas which destroyed poliovirus 1 in a period of several days.

Tarabcak, M., Micekova, M., Sachova, J. (1969). *Sporadic Cases of Pharyngoconjunctival Fever and Follicular Conjunctivitis in Eastern Slovakia in the Years 1962-1968.* **CESK OFTAL, 25(11)**:341-6. Czechoslovakian.

From 1962 to 1968, 155 sporadic cases of pharyngoconjunctival fever and follicular conjunctivitis occured. Adenoviruses were responsible in 77 patients.

Adenovirus 3 was recovered from patients most frequently. Types 1, 4, 5, 6, and 14 were also recovered.

Illnesses occurred primarily in persons more than 15 years old. Nine percent of the cases occurred in children one to 15 years of age, and 14% of the cases occurred in infants. Almost all patients bathed in swimming pools or rivers severals days before onset of illness.

The disease was self-limiting and mild. No corneal injuries occurred.

Thorup, R. T., Nixon, F. P., Wentworth, D. F., Sproul, O. J. (1970). *Virus Removal by Coagulation with Polyelectrolytes.* **J AMER WATER WORKS ASSN, 62(2)**:97-101.

A cationic polyelectrolyte more effectively removed viruses from turbid waters than anionic and nonionic types. Polyelectrolytes acting as primary coagulants were salt dependent. $CaCl_2$ was the most effective salt tested. In instances of poor coagulation, cationic polyelectrolytes exerted a beneficial effect on the formation of flocs and on the removal of virus. Eighty to 94% removal of coliphage T2 and poliovirus 1 were obtained. In instances of adequate coagulation, cationic polyelectrolytes did not increase virus removal beyond the levels obtained with unaided $Al_2(SO_4)_3$ or $Fe_2(SO_4)_3$ coagulation.

Toenniessen, G. H., Johnson, J. D. (1970). *Heat Shocked Bacillus Subtilis Spores as an Indicator of Virus Disinfection.* **J AMER WATER WORKS ASSN, 62(9):** 589-93.

Bacillus subtilis spores heat-shocked at 96C demonstrated altered resistance to chlorination. Increased sensitivity to chlorine paralleled increased exposure to the high temperature. More than 95% of spores heat-shocked for about 7.5 minutes were destroyed in 15 to 30 minutes by one to two mg/liter of HOCl.

Heat-shocked spores of **Bacillus subtilis** may serve as indicator organisms for the destruction of viruses in water.

Turischeva, G. G., Medvedev, M. P., Ivanova, L. P., Kamenskii, V. A. (1969). *Circulation of Enteroviruses in the Sewage of Voronezh in 1966-1967.* **GIG SANIT, 34(9):**119-21. Russian. **(Translation-HYG & SANIT, 34(9):**432-4, 1969.)

From June 1966 through December 1967, 416 sewage samples yielded 134 cytopathic agents (32.2%), mostly coxsackieviruses and echoviruses. Some polioviruses were also recovered.

During this period, 719 specimens from sick children yielded 200 strains of enteroviruses (27.7%) and 286 specimens from healthy children yielded 49 strains (13.6%). Specimens from 150 adult patients yielded seven strains (4.6%). These data suggest that the sources of enteroviruses in sewage are virus carriers among children rather than among adults.

The maximum number of coxsackieviruses (mostly B5) were recovered in summer (end of May through August). From October to November, a second peak occurred. It differed from the summer peak in that it was caused by coxsackievirus B2. Echoviruses occurred primarily in the autumn.

No outbreaks of viral diseases were recorded during this period, although sporadic cases did occur. Coxsackievirus B2 was recovered from both sick and healthy children.

The recovery of specific enterovirus types from patients and carriers and from sewage were temporally related.

Vajdic, A. H. (1970). *Inactivation of Viruses in Water Supplies by UV Irradiation.* **WATER POLLUT CONTR (Ontario Water Research Commission), 108:**24.

In laboratory studies, ultraviolet radiation rapidly inactivated a phage of **Escherichia coli** B, but did not sterilize sewage effluent even after prolonged exposure.

Wachs, A. M. *The Outlook for Wastewater Utilization in Israel.* **"Developments in Water Quality Research," edited by H. I. Shuval. Proceedings of the Jerusalem International Conference on Water Quality and Pollution Research, June 1969. Ann Arbor-Humphrey Science Publishers, Ann Arbor, Michigan, U.S.A. (London, England) (1970),** 109-23.

Important aspects of wastewater reuse are extensively discussed. Experimental studies are described demonstrating removal of poliovirus 1 from stabilization pond effluent treated with a polyelectrolyte, alum, or lime. No virus was detected after lime coagulation, less than 2 PFU/ml after alum treatment, and several hundred PFU/ml after polyelectrolyte treatment. The amount of virus present prior to treatment was almost 10,000 PFU/ml.

Wallis, C., Grinstein, S., Melnick, J. L., Fields, J. E. (1969). *Concentration of Viruses from Sewage and Excreta on Insoluble Polyelectrolytes.* **APPL MICROBIOL, 18(6):**1007-14.

Viruses from sewage were adsorbed on and eluted from an insoluble crosslinked copolymer of maleic anhydride. Viruses either added to sewage or contained in sewage were adsorbed to this polyelectrolyte at a pH of 5.0 to 6.0, and were eluted at pH 8.0 to 9.0.

In a two-month survey of viruses in sewage in April and May 1968, when viruses were at low levels, detection of these agents was accomplished with the polyelectrolyte concentration method. This method lent itself to detection of small amounts of viruses in feces, urine, sewage, and natural waters. Large volumes of fluids could be treated with the polymer, and viruses could be concentrated sufficiently for detection.

Welke, G. (1969). *Virological Studies of a Mechanical Biological Sewage Treatment Plant.* **Z GES HYG, 15(11)**894-7. German.

A mechanical biological wastewater purification plant was sampled for viruses weekly for one year at four different sites which corresponded to

purification stages. Of 168 samples, 76% contained viruses. Sixteen different enteroviruses and adenoviruses were found. Most virus types detected in the influent of the plant were also found in the effluent.

Some virus types isolated from the plant were the same as some isolated from patients in the community.

Wiley, B. B., Westerberg, S. C. (1969). *Survival of Human Pathogens in Composted Sewage.* **APPL MICROBIOL, 18(6)**:994-1001.

After 43 hours of composting, **Salmonella newport**, poliovirus 1, **Ascaris lumbricoides** ova, and **Candida albicans** that had been added to raw sewage sludge were no longer detectable. The poliovirus 1 was most sensitive requiring one hour for complete inactivation, and **C. albicans** was the most resistant, requiring more than 28 hours of composting for complete inactivation. The data from this study indicated that aerobic composting of sewage sludge destroys the pathogens when a temperature of 60 to 70C is maintained for three days.

Wilterdink, J. B., Smit, G. L., Verlinde, J. D. (1969). *Neurovirulence of Polioviruses Isolated from Sewage and Stools of Healthy Children. I. Studies on Neurovirulence, Antigenic Marker and RCT/40 Marker of Type 2 Strains.* **ARCH GES VIRUSFORSCH, 28**:68-76.

Antigenic and rct/40 markers of 56 poliovirus 2 strains were examined. Thirty-nine strains originated from stools of healthy children, and 27 from sewage samples. Seven strains were also tested for monkey neurovirulence.

The seven strains tested for neurovirulence were intermediate between highly neurovirulent and highly attenuated strains. The correlation between neurovirulence and the rct/40 marker was better than between neurovirulence and the antigenic marker.

Wilterdink, J. B., Weiland, H. T., Verlinde, J. D. (1970). *A Longitudinal Study on the Significance of Examining Sewage for the Presence of Polioviruses in the Population.* **ARCH GES VIRUSFORSCH, 32**:82-90.

The distribution of polioviruses in sewage in The Netherlands was studied monthly from 1964 to 1968. Twenty-five sewage treatment plants were examined during the first two years of the study, and 40 plants were examined in the last three years. The resulting data were compared with those obtained from fecal specimens of healthy children in five-day nurseries during the first two years of the study, and from ill persons during the entire study period.

The results suggested that sewage was the best source of information on the circulation of polioviruses in a community. A correlation between the isolation of polioviruses from human individuals and from sewage was observed at times, but there were periods in which such a correlation could not be made, especially when low levels of poliovirus circulation occurred.

Other enteroviruses, reoviruses, and adenoviruses were also recovered during the study.